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Effect of *Raoiella Indica* Hirst (Acari: Tenuipalpidae) Infestation on Chlorophyll and Photosynthetic Efficiency of *Areca Catechue*

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Abstract— *Areca catechu L. is one of the most important plantation crop cultivated in Kerala. Recent reports indicate that phytophagous mites could infest the foliage and nuts of areca palm and induce substantial damage, leading to various levels of economic loss. Among the mites, Raoiella indica Hirst, popularly called Red Palm Mite (RPM) has been recognized as a serious pest of areca palm in various localities of India. Various life stages of this mite were found colonizing and feeding on the lower surface of palm leaves, by de-sapping and which in turn would lead to the formation of yellowish speckles and bronzing in later stages and the ultimate drying up of the leaves. Photosynthesis, being a vital process determining the plant vigour and productivity should be addressed with great concern. In the present study, attention has been focused to analyse the feeding impact of the red palm mite, on the photosynthetic machinery of areca palms through the quantitative estimation of chlorophyll 'a', 'b', 'total chlorophyll' pigments and also 'photosynthetic efficiency' of mite infested and uninfested leaves. Results of the study revealed that the feeding activity of the mite on the leaves of A.catechu induced drastic reduction in the levels of chlorophyll 'a', 'b', 'total chlorophyll' and 'photosynthetic efficiency'. The percentage loss in chl 'a', chl 'b' and 'total chlorophyll' were 62.21±0.63, 57.67±0.73 and 59.884±0.375 respectively in RPM infested Areca leaves. RPM infestation varied significantly in photosynthetic parameters such as minimum fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv), quantum yield of photosynthesis (Fv/Fm), performance index (PI) and the Area which has a negatively impact on photosynthetic efficiency of areca palms. These data obtained from chlorophyll analysis and photosynthetic efficiency were found significant at 0.05level, up on statistical analysis using t-test, thereby establishing the pest status of RPM on A. catcheu.*

Keywords—Raoiella indica; Areca catechu; Tenuipalpidae; chlorophyll; Photosynthetic efficiency; variable fluorescence; quantum yield of photosynthesis.

I. INTRODUCTION

Raoiella indica, a member of the family Tenuipalpidae, reported as a cosmopolitan species infesting a variety of economic crops and it feeds on the underside of palm fronds of various hosts in the orders Arecales and Zingiberales. *R. indica* has been reported as a serious pest of economically important trees like the coconut (*Cocos nucifera*) and banana (*Musa* spp.) (Nagesha-Chandra & Channabasavanna, 1984; Welbourn, 2006 & Sudheendrakumar *et al*., 2010). Arecanut is an important money crop in India and it is cultivated primarily for its kernel obtained from the fruit. An array of insect and non-insect pests are known to infest the stem, leaves, inflorescence, roots and nuts of areca palm, in one or other stages of growth. Of these, *R. indica*, the so called Red palm mite (RPM), is an important sucking pest of young palms, especially in hot, dry weather (Patel and Rao, 1958). Significantly higher population densities of RPM has been recorded on areca palm, with peak population in April/May (Yadavbabu & Manjunatha, 2007). The bottom frond leaves harbour relatively high densities of the mite than the middle and top leaves. RPM forms the first mite species reported to feed through stomata of host plants (Ochoa *et al*., 2011) and this specialized feeding habit would probably impair the photosynthetic and respiratory processes of the host plants. Photosynthesis, being the basis of energy gain in all ecosystems and any reduction in this process would affect the growth and productivity of plants (Zelitch, 1975). Photosynthetic efficiency is determined by various factors, of which the role of chlorophyll is known to be crucial. Photosystem II (PSII) is a good factor to study response and adaptation to abiotic and biotic stress by plants. Considering this, the present work has been taken up to understand the extend of chlorophyll loss and loss of photosynthetic efficiency of infested and uninfested leaves of areca palms by RPM on the areca palms cultivated in Kerala state in India.

II. MATERIALS AND METHODS

Field sampling of mite infested leaves were carried out from areca plantations in North Kerala where palms belonging to six to seven years of age were cultivated in separate gardens from 10 various localities for two months, April and May, 2015. Ten uninfested and infested leaf samples were collected randomly from each locality and kept in separate polythene bags. Samples were transported to the laboratory for subsequent microscopic observation under a Stereo Zoom Microscope (MVNSZ - 450) for recording the presence of various life stages of *R.indica*.

Qualitative and quantitative assessment of the damage induced by *R.indica* on the leaves of *A.catechu* was performed by rearing the species in the laboratory. Live specimens comprising 20–25 adults of *R.indica* were transferred to fresh uninfested leaves of *A. catecheu*. Rearing of live mites were carried out in the laboratory by placing these leaf samples containing mite specimens on moistened cotton pads kept in petridishes. Regular observations were carried out under microscope to record the development of feeding symptoms on the leaf samples. Qualitative analysis of feeding damage at different life stages of *R.indica* was made by observing the development of chlorotic spots and yellowish white patches on the lower surface of the leaf lamina. Regular observations were made for 2 months (April–May) for confirmation of results.

Quantitative analysis of feeding damage was made by estimating the loss of chlorophyll 'a' , 'b' and 'total chlorophyll' pigments in the leaf tissues of *A. catecheu* infested by *R. indica* following method of Arnon (1949). For rating the damage, two categories of leaves were considered, namely heavily infested and uninfested (control). Chlorophyll was extracted from 2g of the leaf sample in 20ml of 80% acetone. The supernatant then transferred to a volumetric flask after centrifugation at 5000 rpm for 5minutes. The extraction repeated till the residue become colourless. Absorbance of the supernatant was read in a Shimadzu UV-VIS spectrophotometer (Model UV – 1601) at 645 nm, 663 nm and 750 nm against the solvent blank of 80% acetone for chlorophyll 'a', 'b' and 'total chlorophyll'.

Leaf damage estimated, quantitatively, by measuring loss in photosynthetic efficiency of the leaves using the Handy Photo synthetic Efficiency Analyser instrument. Different parameters viz. Minimum fluorescence F0, maximum fluorescence Fm, variable fluorescence Fv, Fv/Fm, P Index and Area of infested and uninfested leaves were measured at room temperature on the fully expanded leaves by recording Chlorophyll fluorescence transient.

III. RESULTS AND DISCUSSION

Results of field studies helped to recognize RPM infestation on the lower surface of the leaves of areca palm, near the midrib or veins. On Stereo zoom microscopic observation, the infested leaves collected from the field, disclosed the presence of large number of yellow and white coloured spots. Later these feeding punctures were found coalesced to form light brown coloured areas (Figure.1. B). Similar damage symptoms were found developed by this species on various palms earlier (Flechtmann & Etienne, 2004 & Beard *et al.*, 2012). Feeding activity of large number of various life stages of the mite resulted in the development of localised yellow colouration on leaf lamina (Fig.1.A,C&D). On progressive feeding, these yellow patches coalesced to become bronze coloured, leading to the withering of the leaves. Leaves bearing such symptoms were found to harbour 20–25 adults, 20–30 nymphs and 20–25 larvae of *R.indica*.

In the present study, symptoms of chlorotic patches were evident when population of the mite was high. Such heavily infested leaf tissues, when subjected to quantitative analysis by the estimation of chlorophyll content, revealed drastic reduction in both 'a', 'b' and 'total chlorophyll' contents (Table 1; Figs. 2,3 &4). As shown in Table- 1 and Fig. 2 heavily infested leaves has 0.47± 0.01mg/ gm chlorophyll 'a' content. When compared to unifested leaves, the infested leaves showed 62.21±0.63 % loss of chlorophyll 'a'. The reduction of chlorophyll 'b' in infested leaves could be recorded as 57.67±0.73% when compared to uninfested leaves and the infested leaves had 0.72±0.01mg chlorophyll 'b'/gm (Table.1. & Fig.3.). The reduction of 'total chlorophyll' in infested leaves could be recorded as 59.884±0.375% when compared to uninfested leaves and the infested leaves had 1.180±0.009 mg 'total chlorophyll'/gm (Table.1.& Fig.4.). These data when subjected to statistical analysis (*t*-test) were found significant at 0.05 levels (Table.2).

In the case of uninfested and infested leaves the mean values for Fv/Fm were 0.804 and 0.473 respectively (Table.3.& Fig.5). The low value of Fv/Fm is an indication of plant's stress due to abiotic or biotic factors (Shigeto and Makoto, 1998) and the reduction of Fv/Fm value in infested plants reflects the decrease in PS II activity (Schansker *et al.*, 2006). The area above the fluorescence curve between F0 and Fm is proportional to the pool size of the electron acceptors QA on the Photosystem II and this area reduced in the infested plant (Joliot and Joliot, 2002). The reduced area in the infested leaves (Table 3 & Fig.5.) might be due to blocking of the electron transfer between reaction centers to the quinine pool due to infestation by RPM.

Earlier studies on *Coleus* owing to infestation by another species of tenuipalpid mite viz. *Brevipalpus obovatus* (Meena & Sadana 1983) also could also establish similar loss in chlorophyll contents. Chlorophyll, being one of the

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major parameter for determining the photosynthetic efficiency of the plant, (Maithra & Sen1988) the feeding impact of RPM should be considered seriously for chartering appropriate control strategies.

IV. CONCLUSION

The heavy loss of chlorophyll pigments and the significant variations in the photosynthetic parameters as evidenced during the present study disclosed the potential of *R. indica* to affect adversely the general health, growth rate and biomass of the host plant, *A. catechu* and thereby leading to reduction in the economic utility of the plant.

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TABLES

Table.1: Quantitative loss in Chlorophyll pigments induced by the feeding activity of R. indica on A. catcheu

Chlorophyll	S. No.	Milligram Chlorophyll/gram tissue		Loss in chlorophyll	% chlorophyll loss
		Uninfested	Infested		
Chlorophyll a	1	1.31	0.52	0.79	60.30
	2	1.23	0.45	0.78	63.41
	3	1.22	0.47	0.75	61.48
	4	1.22	0.46	0.76	62.30
	5	1.12	0.41	0.71	63.39
	6	1.41	0.40	1.01	71.63
	7	1.10	0.43	0.67	60.91
	8	1.08	0.56	0.52	48.15
	9	1.30	0.51	0.79	60.77
	10	1.49	0.45	1.04	69.80
Mean ± SEM		1.25±0.01	0.47±0.01	0.78±0.02	62.21±0.63
Chlorophyll b	1	1.90	0.63	1.27	66.84
	2	1.69	0.68	1.01	59.62
	3	1.88	0.75	1.13	59.93
	4	1.67	0.73	0.94	56.56
	5	1.46	0.70	0.76	52.39
	6	1.69	0.73	0.96	56.88
	7	1.80	0.63	1.17	65.14
	8	1.80	0.64	1.16	64.58
	9	1.39	0.80	0.59	42.91
	10	1.85	0.89	0.96	51.83
Mean ± SEM		1.71±0.02	0.72±0.01	1.00±0.02	57.67±0.73
Total Chlorophyll	1	3.201	1.145	2.055	64.210
	2	2.912	1.127	1.785	61.299
	3	3.091	1.221	1.870	60.500
	4	2.883	1.187	1.697	58.840
	5	2.581	1.106	1.475	57.137
	6	3.092	1.121	1.971	63.740
	7	2.885	1.056	1.830	63.412
	8	2.865	1.196	1.669	58.261
	9	2.689	1.299	1.390	51.691
	10	3.335	1.343	1.993	59.747
Mean ± SEM		2.953±0.023	1.180±0.009	1.773±0.022	59.884±0.375

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Table .2: Statistical analysis using t- test

Chlorophyll a		Levene's Test for Equality of Variances		t-test for Equality means						
		F	Sig	T	Df	Sig. (2-tailed	Mean Difference	Std. Error Difference	95% Confidence Interval of the df	
									Lower	Upper
	Equal Variance assumed	6.210	.023	17.352	18	.000	.78200	.04507	.68732	.87668
	Equal Variance not assumed			17.352	11.543	.000	.78200	.04507	.68337	.88063
Chlorophyll b	Equal Variance assumed	3.92	.061	16.458	18	.000	.99500	.06046	.86798	1.12202
	Equal Variance not assumed			16.458	12.893	.000	.99500	.06046	.86428	1.1257
Total Chlorophyll	Equal Variance assumed	6.707	.018	22.811	18	.000	1.773	.07774	1.6099	1.9366
	Equal Variance not assumed			22.811	11.646	.000	1.773	.0774	1.6033	1.9432

Table.3 : Quantitative difference in Photosynthetic parameters induced by the feeding activity of R.indica on A.catechu

Sl. No.	F0	Fm	Fv	Fv/Fm	P index	Area
Uninfested						
1.	662	3274	2612	0.798	0.789	44500
2.	623	3264	2641	0.809	1.38	46800
3.	654	3355	2701	0.805	1.0219	48300
4.	674	3343	2669	0.798	0.784	44300
5.	662	3431	2769	0.807	1.027	47600
6.	634	3454	2820	0.816	1.112	48200
7.	634	3414	2780	0.814	1.048	47800
8.	670	3484	2814	0.808	1.036	46600
9.	663	3523	2860	0.812	1.044	47100
10.	665	3550	2885	0.813	1.072	48100
Mean ± SEM	**654 ± 1.747**	**3409 ± 9.863**	**2755 ± 9.440**	**0.808 ± 0.001**	**1.031 ± 0.017**	**46930 ± 145.453**
Infested						
1.	422	1064	642	0.603	0.119	24000
2.	382	721	339	0.470	0.019	6000
3.	584	969	385	0.397	0.012	7000

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4.	114	170	56	0.329	0.006	12000
5.	106	202	96	0.475	0.014	1000
6.	372	721	349	0.484	0.016	8000
7.	594	969	375	0.387	0.009	10000
8.	114	170	56	0.329	0.007	7000
9.	609	1264	655	0.518	0.057	16700
10.	278	521	243	0.466	0.036	14300
Mean ± SEM	**358 ± 20.100**	**677 ± 39.900**	**320 ± 21.599**	**0.446 ± 0.009**	**0.030 ± 0.004**	**10600 ± 648.742**

FIGURES

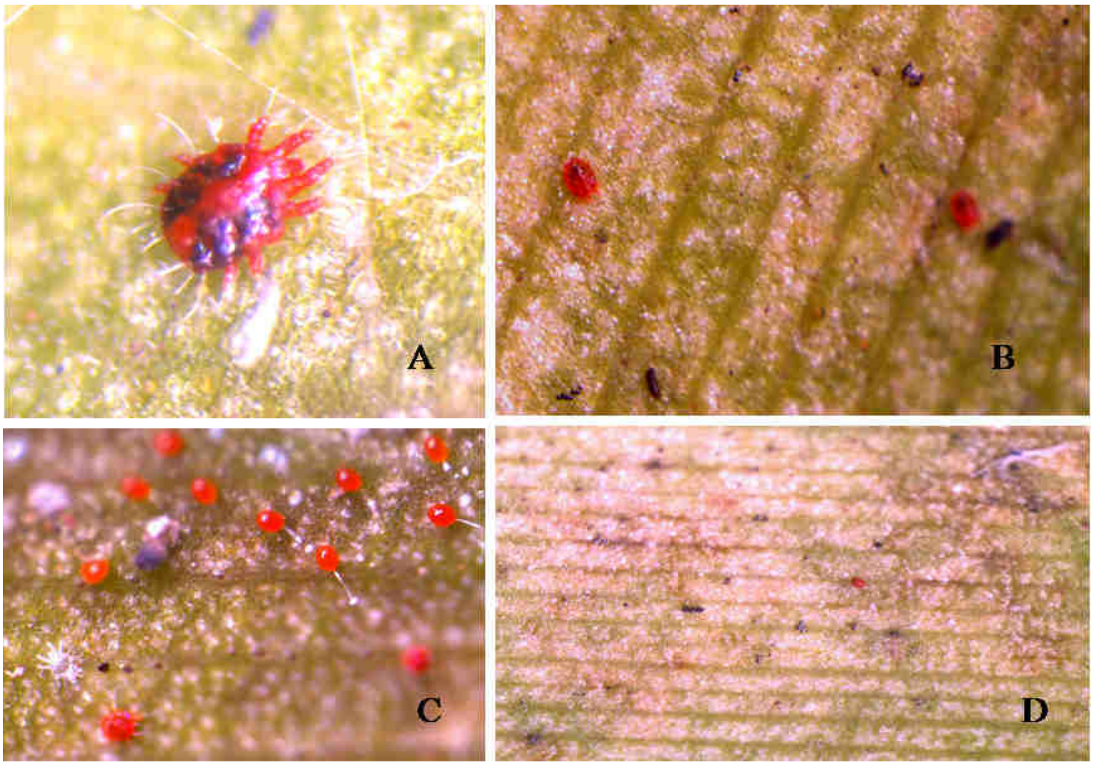

Fig.1: A- Adult female Raoiella indica on areca leaf, B- Infested leaf lamina with R. indica, C- Eggs & larva of R. indica, D – Heavily infested leaf

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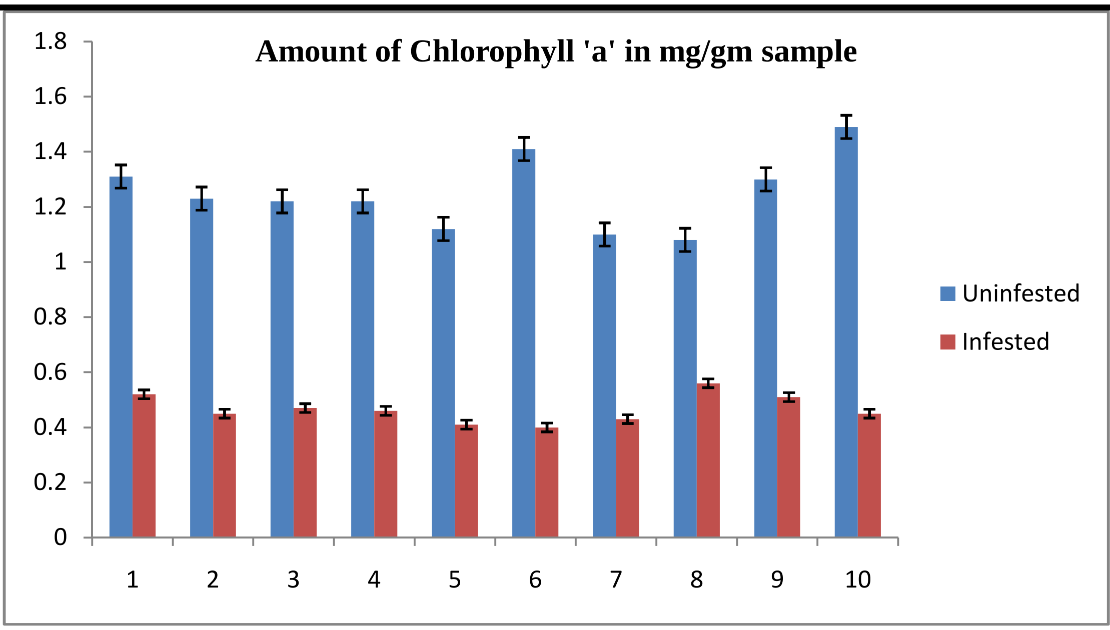

Fig.2: Amount of chlorophyll 'a' in the R. indica infested and uninfested leaf samples of A. Catechu.

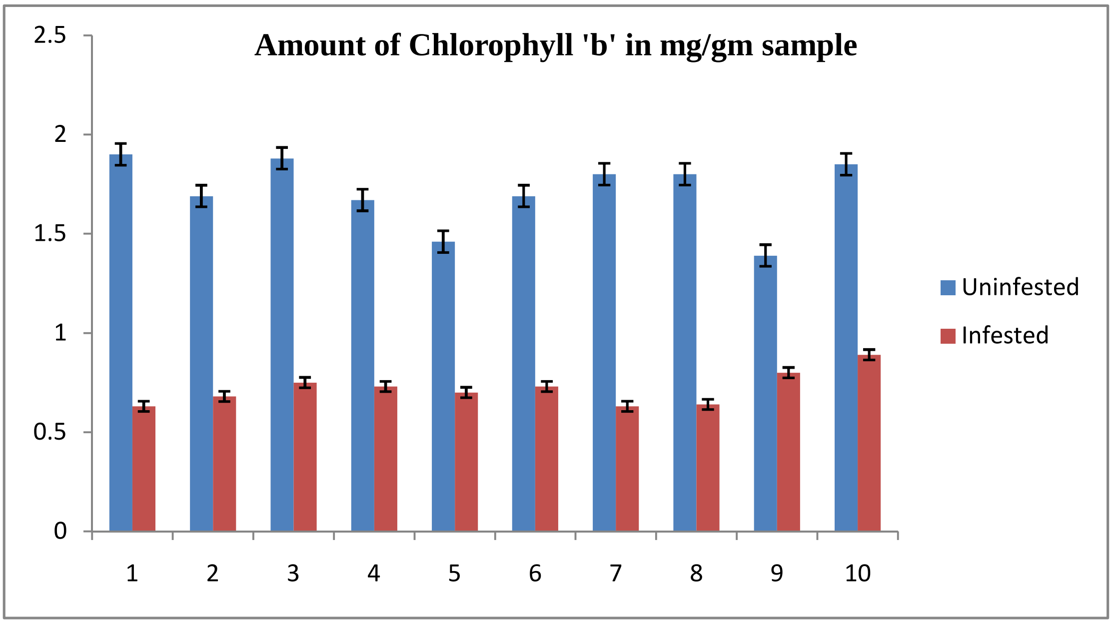

Fig.3: Amount of chlorophyll 'b' in the R. indica infested and uninfested leaf samples of A. Catechu.

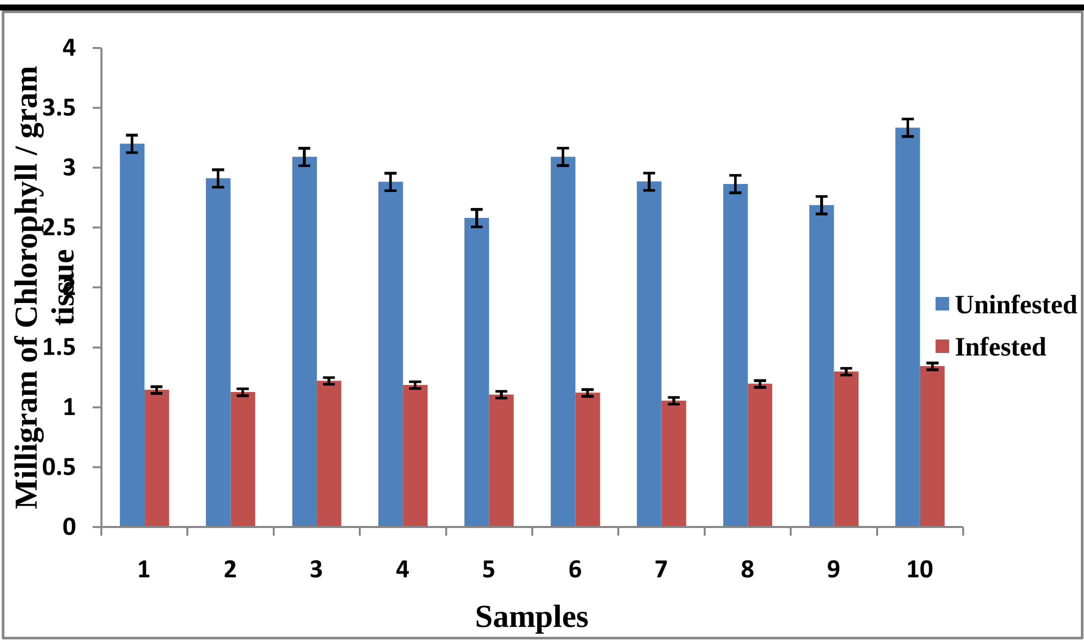

Fig.4.Quantitative loss in total chlorophyll pigments induced by the feeding activity of R. indica on A. catehu

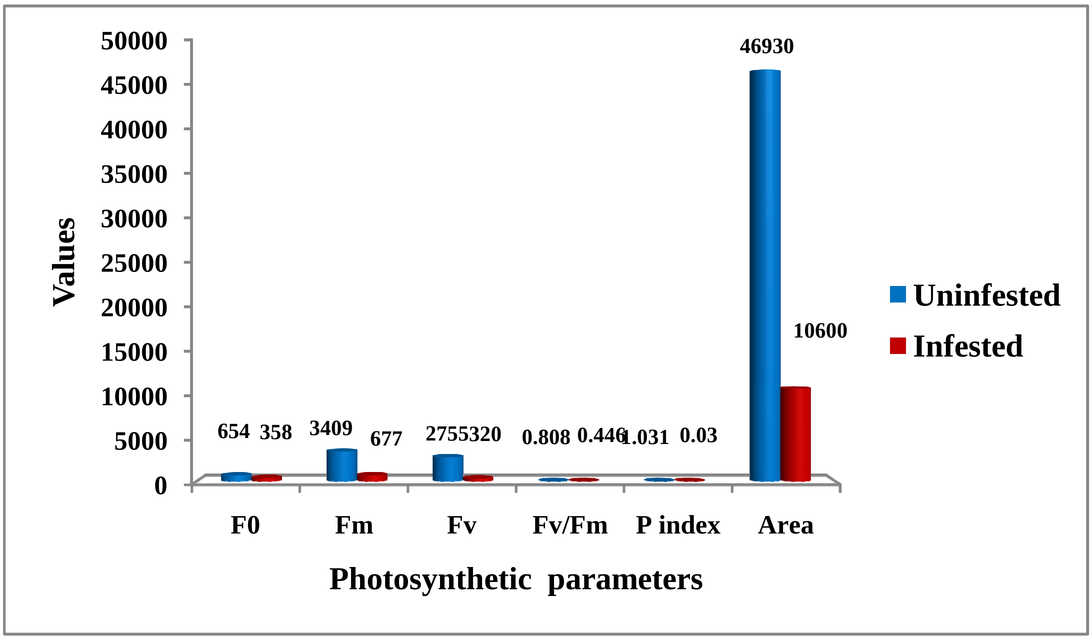

Fig. 5.Quantitative loss in Photosynthetic parameters induced by the feeding activity of R. indica on A. catechu